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(54) Title: THE USE OF PROTEINS EXTRACTABLE FROM ANIMAL ORGANS FOR THE PREPARATION OF MEDICAMENTS FOR THE TREATMENT OF PATHOLOGICAL CONDITIONS CHARACTERIZED BY HYPERPRODUCTION OF TUMOR NECROSIS FACTOR (TNF)

(57) Abstract

Proteins extractable with perchloric acid from mammal liver, in particular from goat liver, are capable of lowering blood levels of Tumor Necrosis Factor (TNF) and can be used for the treatment of multiple sclerosis, rheumatoid arthritis, septic shock and other pathologies characterized by TNF hyperproduction.

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THE USE OF PROTEINS EXTRACTABLE FROM ANIMAL ORGANS FOR THE PREPARATION OF MEDICAMENTS FOR THE TREATMENT OF PATHOLOGICAL CONDITIONS CHARACTERIZED BY HYPERPRODUCTION OF TUMOR NECROSIS FACTOR (TNF)

The present invention relates to the use of proteins extractable from animal organs for the preparation of medicaments for the treatment of pathological conditions characterized by hyperproduction of Tumor Necrosis Factor (TNF).

TNF, also known as cachectin, is a proinflammatory cytokine playing an important role in starting, together with IL-1, the cascade of other cytokines and factors which trigger the immune response in infections and in cancer. This response is paramount for a complete resolution of infections and metastatic processes, but it can occur in an uncontrolled way, thus causing damage to the host. TNF hyperproduction is considered to be involved in a number of pathological conditions, such as septic shock, tumor cachexia, autoimmune diseases (rheumatoid arthritis, multiple sclerosis), meningococcal septicemia, Chron's disease, etc..

WO 92/10197 disclosed protein fractions extractable with perchloric acid from organs of mammals, and their use as anticancer agents. Within these fractions, three main components could be identified, having molecular weights of 50, 14 and 10 KDa on gel electrophoresis. Hereinafter, the purified extract containing these three components will be referred to as UK 101. The sequence of the 14 KDa component, which is the main, if not the only protein, responsible for the described activities,

is reported in WO 96/02567 and it has turned out to be related to that described by other authors (Levy-Favatier, Eur. Biochem. 1903, 212 (3) 665-73) who have assumed that the novel identified sequences belong to the family of the proteins known as chaperonins, to which the HSPs (Heat Shock Proteins) themselves belong.

The proteins described in WO 92/10197 and those of WO 96/02567 (hereinafter referred to as UK 114) show properties not previously observed in chaperonins or analogous proteins. Now it has been found, in particular, that said proteins are capable of significantly lowering TNF blood levels and therefore they can be used for the treatment of pathological conditions characterized by hyperproduction of TNF.

The invention relates particularly to the use of the purified protein UK 114.

Moreover the invention comprises the use of proteins showing high homology to UK 114, of at least 80%, especially of 90% or more.

The activity of the proteins UK 101 and UK 114 has been demonstrated in vitro, on mononuclear leukocytes from peripheral blood and in vivo, by evaluating the effect of the administration of UK 101 on the production by mouse splenocytes as reported hereinafter.

In vitro tests

Mononuclear leukocytes from peripheral blood (PBMC), at a concentration of 1 million/ml, were stimulated in vitro with lipopolysaccharide (100/ ng/ml), for 4 hours, in the absence or in the presence of UK 114 (1 µg/ml and 10 µg/ml).

TNF levels were measured by ELISA.

Results

TNF production by PBMC was inhibited by the addition of UK 114 *in vitro*.

The decrease was by 90% with a 1 µg/ml dose of UK 114 and by 70% with a 10 µg/ml dose of UK 114.

***In vivo* tests**

Treatment:

Mice were treated with 100 µg/mouse of UK 101 on alternate days for 15 days (7 injections).

TNF has been measured 48 hours after the first injection and 48 hours after the last administration.

Preparation of the cells and TNF measurements

Splenocytes (4 x 10^6 cells/ml) were incubated in the presence of 10 µg/ml of the polyclonal mitogen Concanavalin-A (With-A), for 48 hours, at 37°C, 5% CO_2.

The amount of produced TNF released into the supernatant has been evaluated using an immunoenzymatic method (ELISA).

Results

Treatment with UK 101 significantly decreased TNF production by mouse splenocytes. The effect is evident 48 hours after the first administration and it is still present even 48 hours after the seventh administration.

TNF, pg/ml

	physiological saline	UK-101
48 hours after the 1st administration	387 ± 72	247 ± 30°
48 hours after the 7th administration	366 ± 46	264 ± 76,1°

° = p value

Therefore, UK 101 and UK 114 are capable of modifying the course of, or preventing, pathological conditions characterized by TNF hyperproduction, such as multiple sclerosis, rheumatoid arthritis, tumor forms, septic shock, Chron's disease, etc..

The proteins of the invention can be administered by means of suitable formulations, preferably injectable forms.

The procedure of administration (doses, frequency of administration, etc.) will be determined according to the circumstances, depending on a number of factors such as the condition of the patient, stage of the disease. Nevertheless a daily dosage ranging from 1 to 100 mg will be suitable.

TABLE

Met Ser Glu Asn Ser Glu Glu Pro Val Gly Glu Ala Lys Ala
1 5 10

Pro Ala Ala Ile Gly Pro Tyr Ser Gln Ala Val Leu Val Asp
15 20 25

Arg Thr Ile Tyr Ile Ser Gly Gln Leu Gly Met Asp Pro Ala
30 35 40

Ser Gly Gln Leu Val Pro Gly Gly Val Val Glu Glu Ala Lys
45 50 55

Gln Ala Leu Thr Asn Ile Gly Glu Ile Leu Lys Ala Ala Gly
60 65 70

Cys Asp Phe Thr Asn Val Val Lys Ala Thr Val Leu Leu Ala
75 80

Asp Ile Asn Asp Phe Ser Ala Val Asn Asp Val Tyr Lys Gln
85 90 95

Tyr Phe Gln Ser Ser Phe Pro Ala Arg Ala Ala Tyr Gln Val
100 105 110

Ala Ala Leu Pro Lys Gly Gly Arg Val Glu Ile Glu Ala Ile
115 120 125

Ala Val Gln Gly Pro Leu Thr Thr Ala Ser Val
130 135

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Zetesis s.p.a.

(B) STREET: Galleria del Corso 2

(C) CITY: Milano

(E) COUNTRY: Italy

(F) POSTAL CODE (ZIP): 20122

(ii) TITLE OF INVENTION: The use of proteins extractable from animal organs for the preparation of medicaments for the treatment of pathological conditions characterized by hyperproduction of tumor necrosis factor (TNF)

(iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 137 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Ser Glu Asn Ser Glu Glu Pro Val Gly Glu Ala Lys Ala
1 5 10

Pro Ala Ala Ile Gly Pro Tyr Ser Gln Ala Val Leu Val Asp
15 20 25

Arg Thr Ile Tyr Ile Ser Gly Gln Leu Gly Met Asp Pro Ala
30 35 40

Ser Gly Gln Leu Val Pro Gly Gly Val Val Glu Glu Ala Lys
45 50 55

Gln Ala Leu Thr Asn Ile Gly Glu Ile Leu Lys Ala Ala Gly
60 65 70

Cys Asp Phe Thr Asn Val Val Lys Ala Thr Val Leu Leu Ala
75 80

Asp Ile Asn Asp Phe Ser Ala Val Asn Asp Val Tyr Lys Gln
85 90 95

Tyr Phe Gln Ser Ser Phe Pro Ala Arg Ala Ala Tyr Gln Val
100 105 110

Ala Ala Leu Pro Lys Gly Gly Arg Val Glu Ile Glu Ala Ile
115 120 125

Ala Val Gln Gly Pro Leu Thr Thr Ala Ser Val
130 135

CLAIMS

1. The use of proteins extractable with perchloric acid from mammal liver, for the preparation of medicaments for the prevention and the treatment of pathologies characterized by TNF hyperproduction.

2. The use according to claim 1, wherein the protein has the following sequence:

Met Ser Glu Asn Ser Glu Glu Pro Val Gly Glu Ala Lys Ala
1 5 10

Pro Ala Ala Ile Gly Pro Tyr Ser Gln Ala Val Leu Val Asp
15 20 25

Arg Thr Ile Tyr Ile Ser Gly Gln Leu Gly Met Asp Pro Ala
30 35 40

Ser Gly Gln Leu Val Pro Gly Gly Val Val Glu Glu Ala Lys
45 50 55

Gln Ala Leu Thr Asn Ile Gly Glu Ile Leu Lys Ala Ala Gly
60 65 70

Cys Asp Phe Thr Asn Val Val Lys Ala Thr Val Leu Leu Ala
75 80

Asp Ile Asn Asp Phe Ser Ala Val Asn Asp Val Tyr Lys Gln
85 90 95

Tyr Phe Gln Ser Ser Phe Pro Ala Arg Ala Ala Tyr Gln Val
100 105 110

Ala Ala Leu Pro Lys Gly Gly Arg Val Glu Ile Glu Ala Ile
115 120 125

Ala Val Gln Gly Pro Leu Thr Thr Ala Ser Val
130 135

3. The use according to claim 1, wherein the proteins used have a homology of at least 80% to the protein of claim 2.

INTERNATIONAL SEARCH REPORT

Inte Jonal Application No
PCT/EP 98/01516

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 02567 A (ZETESIS SPA) 1 February 1996 cited in the application see page 1 - page 5 --- -/--	1-3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of theinternational search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 119, no. 7, 16 August 1993 Columbus, Ohio, US; abstract no. 65937, XP002075248 & F LEVY-FAVATIER ET AL.: "Characterization, purifiication and cDNA cloning of a rat perchloric-acid-soluble 23 kDa protein present only in liver and kidney " EUR. J. BIOCHEM., vol. 212, no. 3, March 1993, pages 665-673, cited in the application see abstract	1-3
Y	WO 96 10039 A (PEPTIDE THERAPEUTICS LIMITED) 4 April 1996 see the whole document	1-3
Y	GB 2 251 186 A (GATZ & BROMLEY) 1 July 1992 see the whole document	1-3
E	WO 98 11909 A (ZETESIS) 26 March 1998 see the whole document	1-3

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intl Application No
PCT/EP 98/01516

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